Viability of frozen algae used as food for larval penaeids

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Freezing with added chemicals as flocculants and protectants as a means of preserving stock cultures was tried with four algal species commonly used as larval food. They included *Chaetoceros calcitrans, Skeletonema costatum, Tetraselmis chuii* and *Isochrysis galbana.* Except in *I. galbana,* this method successfully preserved the viability of the algae tested.

Freezing as an excellent preservation technique has long been recognized and practiced. It has its early applications in the food industry (Enochian, 1978; Rasmussen, 1968; Persson, 1975); it is also extensively used in the field of medicine for preserving the viability of cells, tissues, and organs (Huggins, 1975; Sell et al., 1975). In aquaculture, freezing also finds its applications. Frozen diatoms are fed to shrimp larvae in Galveston, Texas (Brown, 1972) in Aquacop, Tahiti (Aquacop, 1977) and in SEAFDEC, Iloilo (Millamena and Aujero, 1978). However, as a means of preserving the viability of algae, available data on the subject is scarce although it has been recommended as a means of maintaining algal cultures in a viable state for long periods of time (Holm-Hansen, 1973).

This study was undertaken to assess the effect of simple freezing on the viability of four marine planktonic algae commonly used as food for larval penaeids and to determine the maximum storage effectivity of the preservation technique for each species. It is hoped that results would provide useful information on how to maintain a constant supply of stock cultures in the freezer all year round rather than depend on live algal cultures which are so much affected by weather conditions.

Cultures maintained live by the Phycology laboratory were harvested in their log phase by chemical flocculants: alum, lime or NaOH. The harvested slurries were frozen with or without cryoprotectant (DMSO or glycerol) at -20 to -22^{o}C.

At set time intervals frozen samples were tested for viability by actual reproduction of cells in culture. These were set up in one-liter baxter bottles in a randomized complete block design with treatments representing the harvesting flocculant and cryoprotectant used and whether it was added or not. Fresh culture of the species being tested was used as the control. The experiments were done indoors at 21^{o}C – 24^{o}C and provided with continuous aeration and illumination. Growth was observed by daily monitoring of population densities with a hemacytometer; *C. calcitrans* and *S. costatum* until peak growth, *I. chuii* and *I. galbana* up to one week.

Results show that, except *I. galbana,* freeezing with chemicals and flocculants successfully preserved the viability of the algae tested. *C. calcitrans* was viable up to eighteen months storage; all treatments showing regrowth with no significant difference between peak growths including the control (Fig. 1).

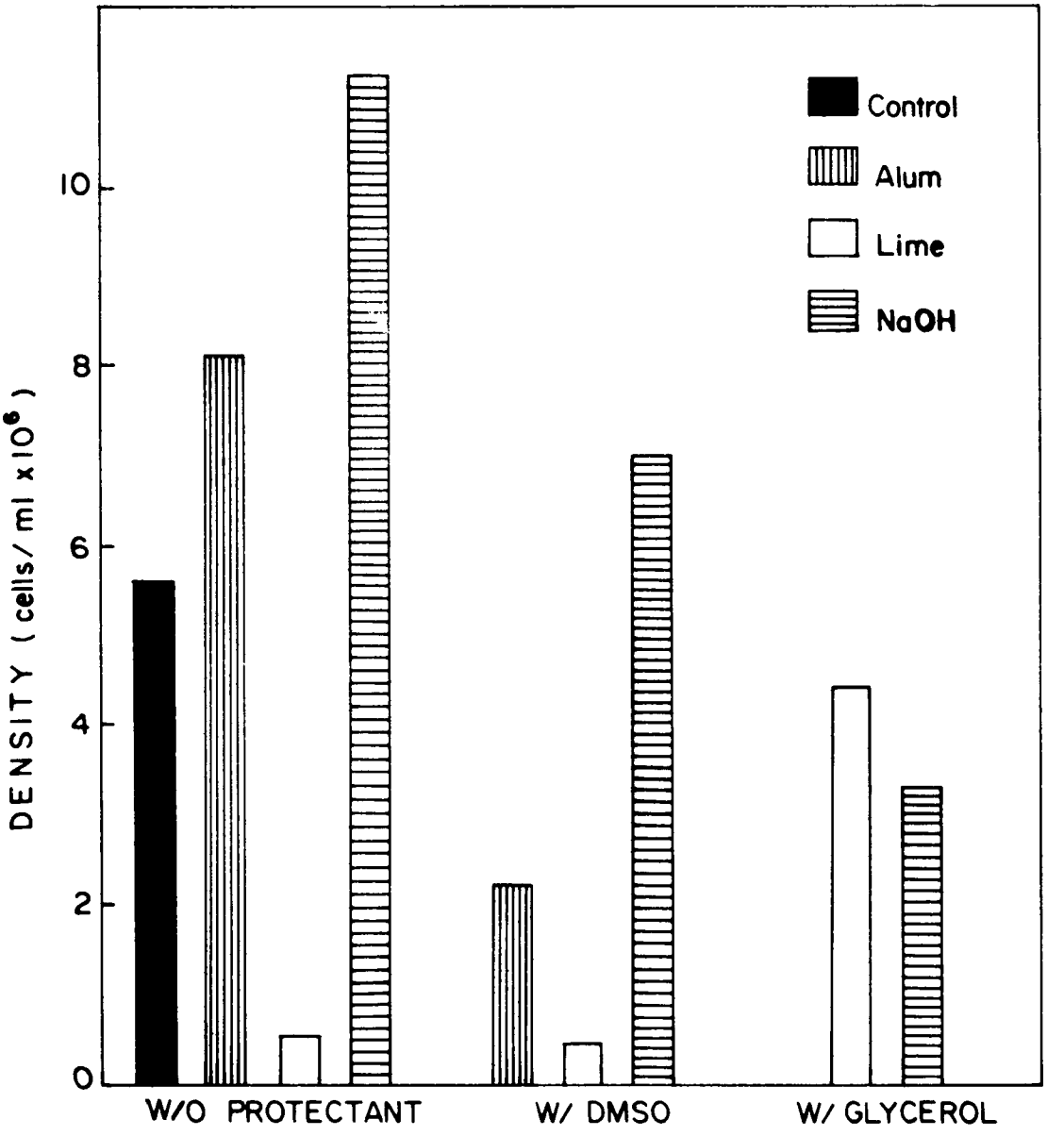

Fig. I. Peak growth attained by *C. calcitrans* frozen for eighteen months (based on 2 replicates).

A comparison of the growth curves of samples stored for different lengths of time showed that the longer the storage time, the longer it takes for the organism to show signs of growth and reach peak growth (Fig. 2). Treatment with alum and glycerol was not included due to lack of sample. The maximum storage effectivity for this species has not been determined hence extended studies have to be done on samples stored beyond eighteen months.

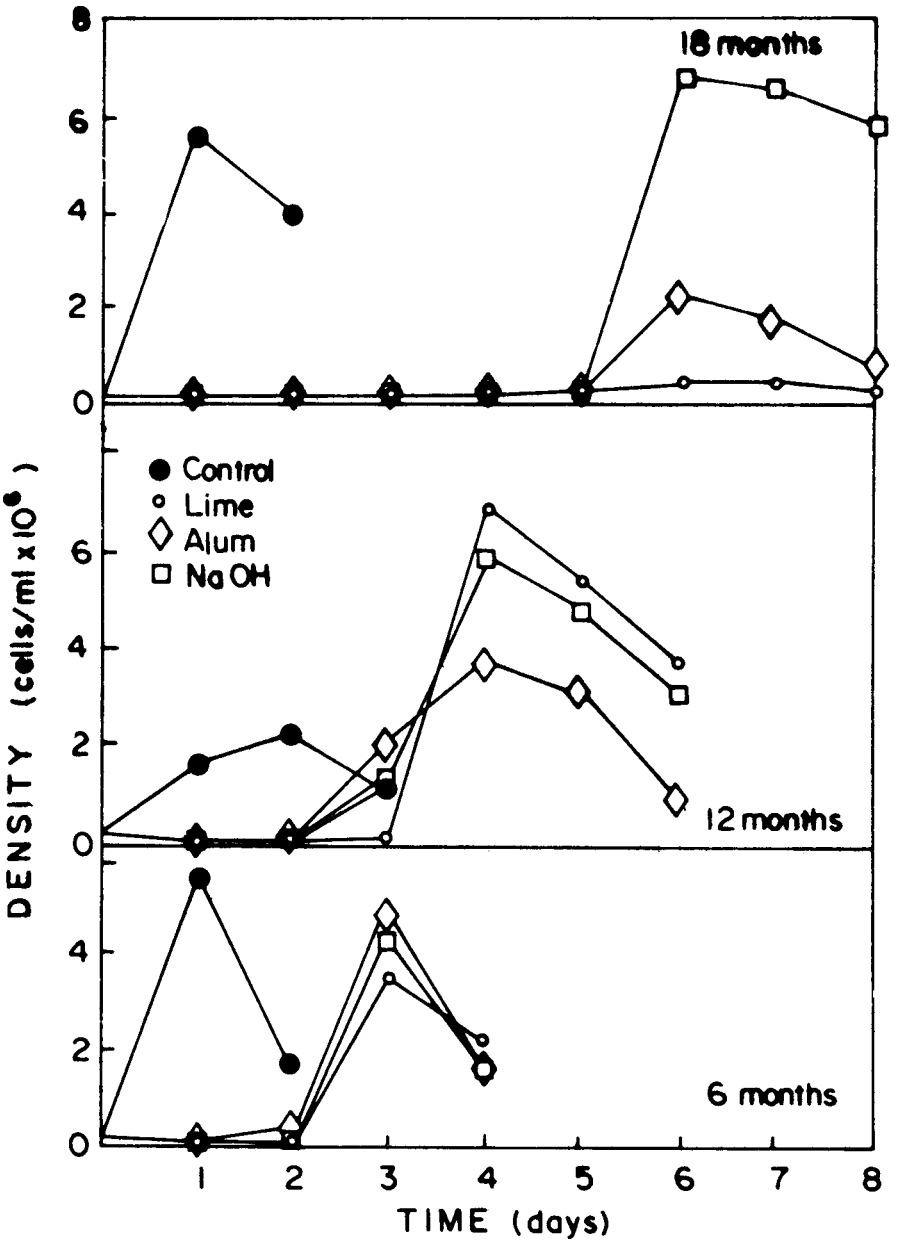

Fig. 2. Growth curves of *C. calcitrans* frozen with DMSO. (6 months, based on 3 replicates, 12 months, unequal replicates).

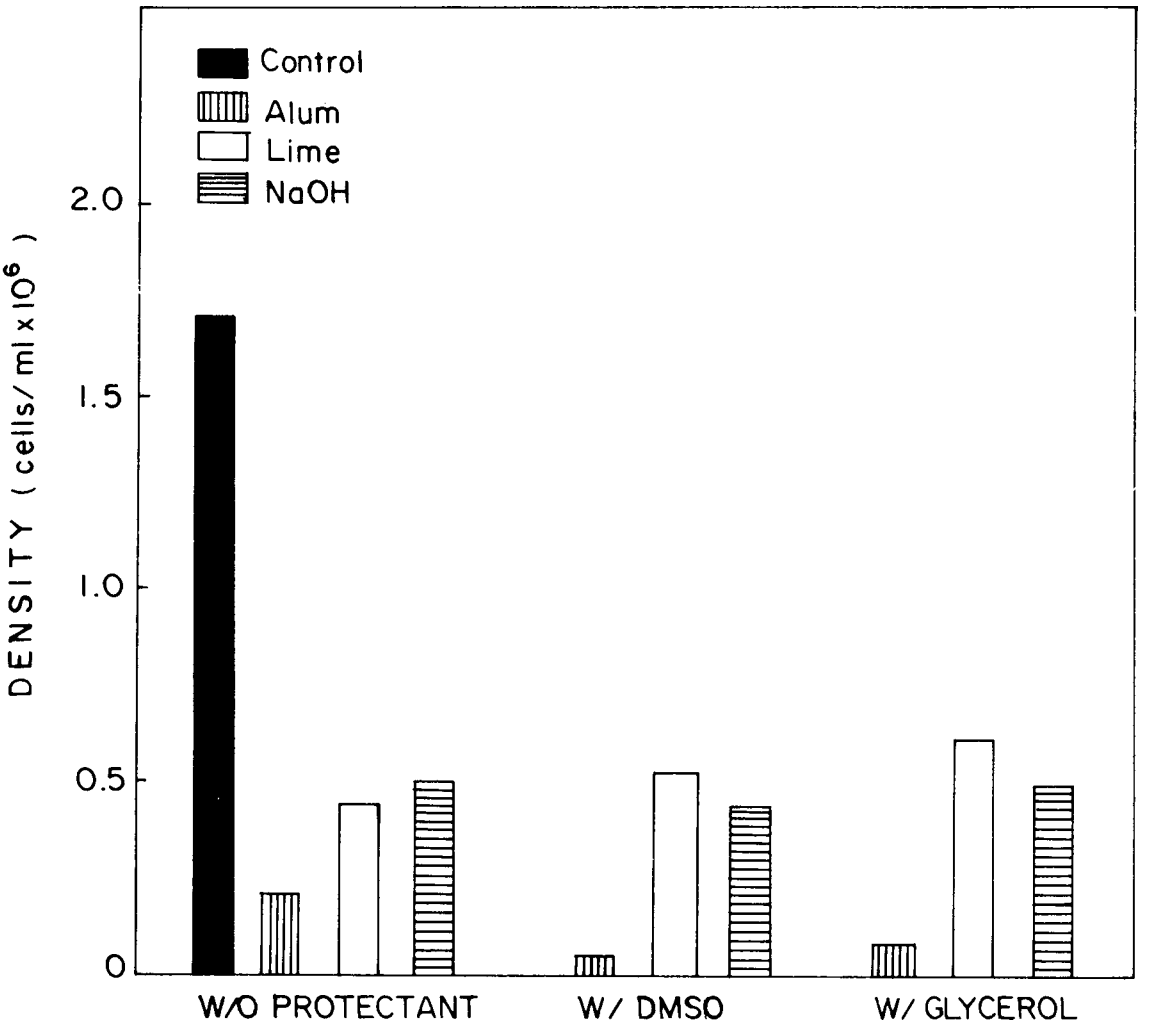

Fig. 3. Peak growth attained by *S. costatum* frozen for two months (based on 3 replicates).

Alum seems to have an adverse effect on *S. costatum* (Fig. 3). All treatments showed regrowth after two months storage; however, a look at their population densities showed the control reached significantly higher peak growth at 10% level. No growth was observed beyond two months.

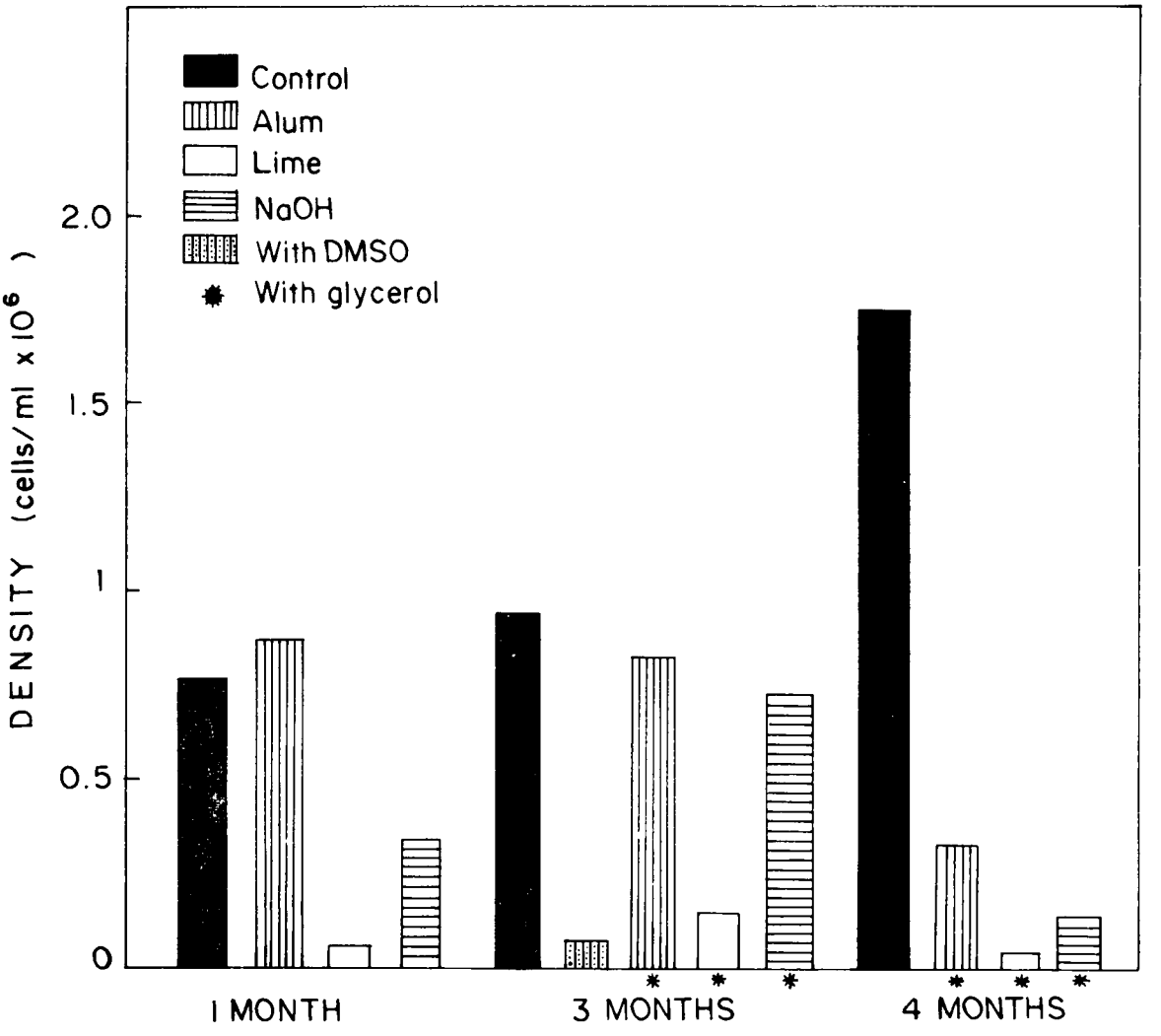

Fig. 4. The effect of cryoprotectants on the viability of *T. chuii* (based on 3 replicates)

Literature cited

Without protectant, *T. chuii* is not able to survive freezing beyond one month. With glycerol it was viable up to four months (Fig. 4). There were no significant differences in population densities attained by cultures that showed regrowth stored for one to three months including the control; however, between treatments that were viable after four months storage, the control gave a significantly higher population density at 10% level. Samples stored beyond four months were not viable.

For this study cryophylaxis did not seem to greatly increase the viability of frozen cells except in *T. chuii;* but for the diatoms viability was preserved regardless of the harvesting flocculant used and whether or not protectants were used.

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